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From Coagulation to Oral Surgery Application: Platelets in Bone Regeneration

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Abstract

The complexity of the treatment of tissue lesions, particularly bone lesions, in regenerative medicine depends on the origin of the substance loss (traumatic, tumoral, infectious, etc.), its size and mechanical requirements. In the field of dental surgery, the need to ensure rapid regeneration of injured bone tissue for periodontal, post-extractional or pre-implant corrective surgery leads dental surgeons to have a large number of biomaterials in their therapeutic arsenal. The mineral materials are most often used because of their chemical composition which is close to bone's mineral phase. They also present a resorption time in agreement with the time of formation of new bone.

However their benefits are inconstant and the need of new bioactive structures, well accepted by the host, and favoring tissue healing has grown. Here is the place for platelet concentrates such as Platelet Rich Plasma (PRP) and Platelet Rich Fibrin (PRF) which are rich in growth factors, cytokines and others proteins. PRF became the most commonly used in the last decade as it is easier to handle with its polymerized form which mimics an extracellular matrix favorable to cell proliferation and differentiation. A new option, called platelet lysate, has recently been highlighted in the general field of tissue regeneration and has the advantage of making platelet's content directly available. Proteins concentrations are increased in these products even if their liquid form complicates their use in daily practice. This mini-review sums up the main clinical interests for the use of platelet concentrates and the new perspectives in the field of alveolar bone regeneration especially with platelet lysate.

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Introduction

Blood-derived materials constitute a family of biomaterials designed from blood components and extracted from patient samples. They may concern only one molecule as is the case of albumin [1] and immunoglobulins [2], or a whole part of the blood sample combining the different elements including cells [3]. The samples may be autologous for an implantation in the same subject or may come from different individuals or even species, provided that the samples do not contain pathogens that would be transmitted. Blood richness in cellular and protein elements offer perspectives in the treatment of pathologies for which healing processes are deficient. Indeed, the presence of elements such as growth factors and cytokines can re-initiate healing pathways by modifying the activity of cells responsible for tissue regeneration.

In the field of dentistry, a wide variety of hard and soft tissues can be affected by acute or chronic destructive diseases. More than dental enamel and dentine which can be affected by caries [4], alveolar bone can be destroyed in periodontitis [5] or periapical infections [6], and the gingival level reduced in periodontitis conditions as well. Cell competition from different tissues to occupy the site to be healed [7], as well as the presence of large numbers of bacteria [8,9] on a complex and weakened immune field [9], complicates scarring processes. The latter must therefore be clinically re launched and the dental surgeon has at his disposal several biomaterials to graft in affected defects [10]. Mineralized biomaterials called bone substitutes may help to enhance alveolar bone volume but results are inconstant and patient dependent [10-12], so here is the place for the use of platelet concentrates, a family of blood-derived materials for transplantation in patients. The first were described in the early 1970's but main advances in their preparation and applications were published in the late 1990's [13].

Thus, the objective of this mini-review is to present actual main clinical interests for the use of platelet concentrates and the new perspectives in the field of alveolar bone regeneration.

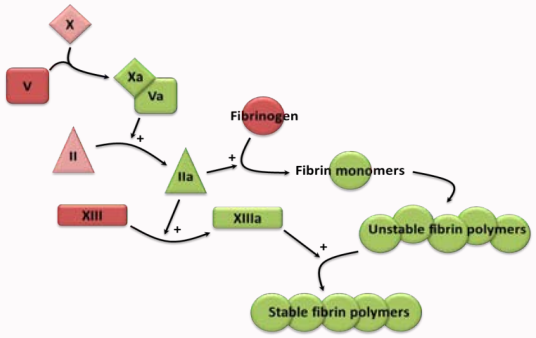

Figure 1: Coagulation pathways involving fibrin and resulting in clot stabilization. Proaccelerin, also called labile factor (noted factor V), binds with Stuart factor (X) to form a complex activating both molecules (Va and Xa). Together, they allow formation of thrombin (IIa) from pro-thrombin (II), the new molecule which plays a dual role of transforming fibrinogen molecules into fibrin monomers and activating fibrin stabilizing factor (XIIIa). This one, as its name indicates, allows the stabilization of fibrin polymers constructed from fibrin monomers previously formed.

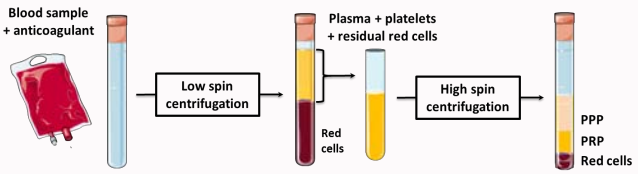

Figure 2: Main method for PRP preparation. After blood sampling and addition of an anticoagulant, a low speed centrifugation (3000 g, 10 min) is performed. Red blood cells are excluded and then a second centrifugation (high speed, between 6000 and 10000 g, 30 min) is applied. It separates platelet rich plasma (PRP) from Platelet Poor Plasma (PPP) and red cells.

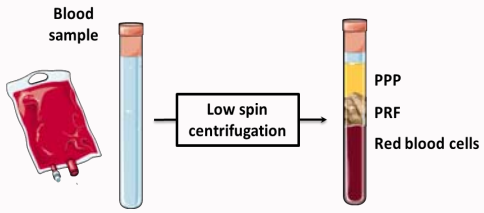

Figure 3: Main method for PRF preparation. Immediately after blood sampling, a low speed centrifugation (3000 rpm, 10 min) is performed without addition of an anticoagulant. It separates platelet rich fibrin clot (PRF) from Platelet Poor Plasma (PPP) and red cells.

Platelets in coagulation pathways

Platelets, also called thrombocytes, are nucleus-free cells resulting from megakaryocyte fragmentation in bone marrow and involved in the phenomena of coagulation [14]. The molecular, cellular and tissue mechanisms recruited during a vascular breach are complex. Briefly, to sum up the implication of fibrinogen and fibrin, when a blood vessel wall is broken, a contact appears between its outer layer and blood, then one molecule in particular, called Tissue Factor (TF), binds blood factor VII and activates it. A complex is thus formed between the TF and the newly created factor VIIa ("a" for "activated") [15]. This complex activates the factors IX and X present on cell membranes, which corresponds to the initiation of the exogenous pathway of coagulation [16-18]. Factor Xa binds to factor Va (activated elsewhere). This causes the platelet wall factors II and X to be activated in turn. Factor IIa appears: it is called thrombin. It has the role among others to ensure the conversion of fibrinogen (soluble, 450 Ångstrom, molecular weight 350 kDa [19]) to fibrin (insoluble) [20,21] by cleaving N-terminal ends of the Aα and Bβ chains of fibrinogen [19]. This allows the release of fibrin monomers and short peptides called fibrin peptides A and B. An association occurs between the newly revealed N-terminal ends on the monomers with the neighboring sequences of the others monomers [22,23]: there is formation of a fibrin polymer. Thrombin also activates the factor XIII, activation that occurs under the influence of calcium whose presence is essential for pro-coagulant complexes formation and thrombin generation [24]. Factor XIIIa is a transglutaminase type enzyme whose roles are to create covalent bonds between the γ chains of several monomers, to create also covalent bonds between the α chains of several monomers and to link the newly formed clot with sub endothelial proteins. All these pathways among the whole process of coagulation lead to clot stabilization [15,25]. A brief sum up of mechanisms involving fibrin is presented on Figure 1.

PRP and PRF in oral surgery

Blood circulating platelets are thus mainly involved in the whole process of coagulation and represent an essential target in biomedicine. In this way, they can be easily isolated from blood samples on citrated tubes and grouped together in products called Platelet Concentrates (PC) that are suitable for clinical use. This PC family especially includes, among others, liquid plasma called Platelet Rich Plasma (PRP) [26] and a fibrin clot formed in absence of anticoagulant called Platelet Rich Fibrin (PRF) [27]. They contain the sample's platelets and a majority of the white cells [26]. Before using them in various biomedical applications, it is possible to ensure that the platelet derivative will no longer contain any pathogens [28], that might have colonized it in the event of contamination [29], with a phase of leucocyte depletion by filtration or other mechanisms such as irradiation. Techniques for the preparation of platelet concentrates are numerous [28], many institutions having designed their own protocol with high levels of efficiency and a good reproducibility of results [30-32]. The PRP isolation method (Figure 2) consists in a two-step gradient centrifugation of a mix containing whole blood and an anticoagulant (*e.g.* acid citrate dextrose). The first centrifugation (soft spin 3000 g, 10 min) results in a leuko reduced platelet-rich plasma supernatant and red blood cells. Then, the second centrifugation (hard spin between 6000 and 10000 g, 30 min) is performed to isolate residual erythrocytes at the bottom and two other phases: Platelet Poor Plasma (PPP) at the top and Platelet Rich Plasma (PRP) in the middle [26]. The objective is to concentrate the platelet pellet into a small amount of plasma volume [33].

In the case of PRF, the main protocol works with low speed centrifugation (3000 g, 10 min) immediately after blood sampling without the use of anticoagulants additives [27,34]. PRF clot appears in the middle of the tube (Figure 3) between platelet poor plasma and red blood cell pellet [27] (Figure 3).

These two P Care growth factors and cytokines rich like VEGF (Vascular Endothelium Growth Factor), PDGF (Platelet-Derived Growth Factor), EGF (Epidermal Growth Factor) or TGF-β (Transforming Growth Factor-beta) [26]. These molecules contributing to involve tissue growing and organ development, it constitutes an important argument for biomedical assays of platelet concentrates [28]. Main applications concern wound healing and musculoskeletal diseases [3] especially in the field of oral surgery, in which the practitioner has to deal with several diseases on alveolar bone and others various tissues, the whole in an infected environment.

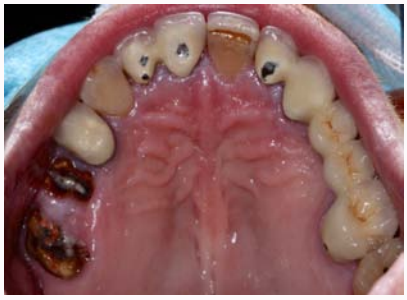

Figure 4: Pre-surgical photography. Teeth number 15 and 16 need to be extracted.

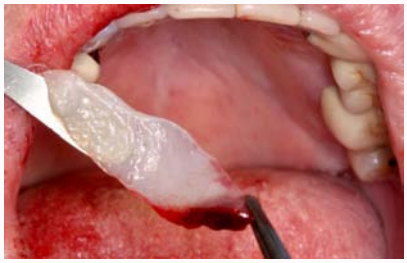

Figure 5: Platelet Rich Fibrin clot obtained after centrifugation and ready to be grafted in extraction sockets.

Oral surgical procedures concern tooth extractions, implantology and period ontology, *i.e.* hard and soft tissue management in healthy or pathologic situations. In a healthy patient, buccal wound would heal thanks to the presence of a simple clot, rich in platelets. However, in some diseases healing process is not efficient. In periodontitis, aggressive bacteria have colonized periodontal tissues and lead to tissue destruction [5]. The process has to be stopped by bacteria elimination but when tissue defect is too important a biomaterial graft may become necessary to help bone regeneration and volume reconstruction. In bisphosphonate-related osteonecrosis of the jaw, bisphosphonate inhibit osteoclasts functions, especially their angiogenic ability [35], and lead to bone necrosis in case of a trauma [36]. In these two examples, wound process has to be re-initiated so here is the main argument favoring the use of platelet concentrates, rich in growth factors, as therapeutic or even preventive materials. In the 2000's, before the increase for interest on PRF, PRP was the main PC to be used in dentistry for bone regeneration in extraction sockets and also in association with other biomaterials for periodontal treatments [37]. It presents the advantage of concentrating a high quantity of autologous platelets in a small volume of plasma but such a liquid is difficult to handle [37]. A mix of PRP with calcium chloride and thrombin leads to platelet activation, growth factors release and material gelation which become easier to manipulate [38].

When the protocol to produce PRF was described it enabled to directly obtain a gelled platelet concentrate with a unique centrifugation of blood sample [34]. Extra-cellular fibrin has polymerized and constituted a tri-dimensional network. Procedure is very simple and no additive agent is necessary to make PRF so the method began to expand in dental practice and gradually over passed PRP in oral surgery [13]. PRF has various properties on cells and tissues, for example it has already been shown that it was able to promote angiogenesis, mitogenesis or cell migration [13]. However PRF and PRP content are very similar and the difference essentially resides on their consistence. Indications between the two

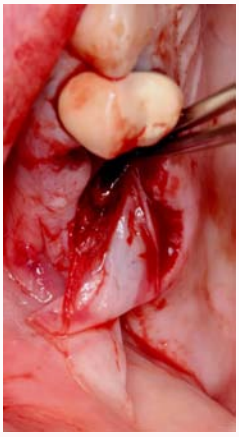

Figure 6: PRF clot is carefully set up into extraction sockets to avoid its fragmentation.

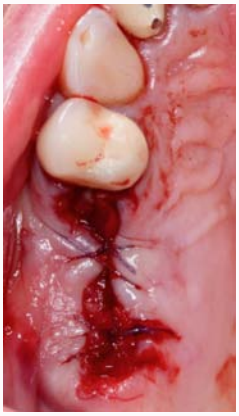

Figure 7: Final sutures must fix vestibular and palatal gum in order to stabilize PRF and blood clots over alveolar bone.

are quite similar, from post-extraction socket filling [39] to guided bone regeneration and soft tissue management [13]. For example, in case of teeth extractions on a patient suffering from a hereditary osteogenesis imperfect, it helps to guide healing process. The patient was treated with intravenous bisphosphonates for about twenty years and stopped her treatment more than ten years ago. She already had previous extractions after this treatment and developed each time an alveolitis. The day of the extraction of teeth number 15 and 16 (Figure 4), a PRF technic was used. The blood sample was centrifuged during the surgery and the PRF clot (Figure 5) was set up into bone socket (Figure 6). Sutures must be performed in order to maintain the clot under the gum and over the bone to heal (Figure 7). Thanks to this technic; the patient's cells are guided in the fiber network in presence of all growth factors. After 2 and 3 weeks (Figures 8,9) final healing was satisfying without any complications; soft tissue maturation was optimal.

A wide perspective from platelet concentrates to platelet lysate

Platelet Lysate (PL) corresponds to platelet's cytoplasm content recovered after destruction of the plasma membrane. Growth factors, fibrinogen molecules, cytokines and other proteins become released and directly accessible. To generate platelet lysate, different methods exists: a thrombin supplementation to initiate thrombocyte degranulation, PC sonication or a frozen sequence to lyse cell membranes [28]. None separation is necessary before protein use,

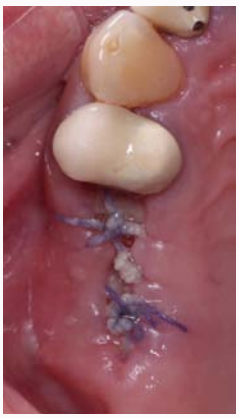

Figure 8: healing after 2 weeks.

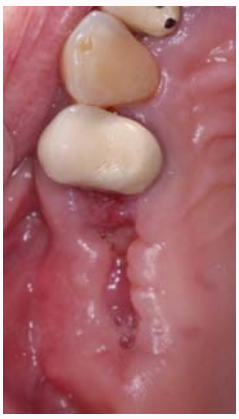

Figure 9: Healing after 3 weeks.

even if a new centrifugation (1400g, 10min) is possible to eliminate cell debris [40]. Fibrin for example, which is already found in platelet concentrates before cell lysing, sees its concentration growing in the lysate. Several platelet lysates obtained from different subjects may then be pooled to grow available quantities before biomedical applications [28] and to optimize the lysate quality. Indeed, it has been shown that the PL's properties on cell proliferation and osteoblastic differentiation were higher with a sample obtained from a younger donor [41]. These pooled human Platelet Lysates (hPL) does not present any risk of immune rejection or pathogen transmission due to filtrations and treatments they have received during their creation protocol.

It is already used routinely *in-vitro* for cell culture medium's supplement [42], especially for cells to be grafted in human because, unlike Fetal Bovine Serum, it has the advantage of being autologous or allogeneic. In the field of dentistry, even if platelet concentrates such as PRP or PRF are currently used for oral surgery, platelet lysate has not been validated yet for a clinical use. Several human studies have already been performed for others biomedical applications, trying to apply PLcutaneous and tissue wounds [27,43] or mucositis [44]. Results on soft tissues are nuanced in comparison with others PC but the main difference in PL is the higher availability of growth factors and other proteins after cell lysis so the expected benefits in bone regeneration are significant. Indeed, when platelet lysate, with its high content in fibrinogen, is associated with coagulation promoters - such as calcium chloride - to create a three dimensional hydrogel [45], it is supposed to generate a PRF-like network with higher availability for growth factors. Inside this structure, human cells would survive and proliferate optimally [46] thanks to the high PL's biocompatibility. However, fibrin network may be degraded too fast [47] and does not present a high resistance to mechanical stress. Associations with other biomaterials should be further studied, especially mineralized bone substitutes (hydroxyapatites, tri-calcium phosphates…), because, even if the benefits on tissue resorption and formation does not seems to be increased [48-50], it would improve the material resistance. Moreover, regenerative therapies must be designed to optimize the potential of surrounding cells, especially in dentistry where the use of cell-graft is restricted. The main limits are put by regulatory frameworks that restrict in many countries the use of exogenous cells and growth factors for human therapy, especially in the context of pathologies with low morbidity. Here is an advantage for the use of autologous platelet lysates, a bioactive structure which would perfectly meet the objective of graft colonization by blood vessels and host's cells [45,51-53].

Conclusion

With the development of platelet concentrates use in all fields of research, the new trend is to study platelet lysate, which is no more complex to obtain than PRP and PRF. Its clinical indications have yet to be clearly defined in large-scale trials, but its *in vitro* benefits, particularly for cell culture, are now well known. The future for the use of this platelet derivative may resides on the treatment of various diseases assimilated to chronic scarring defects because preliminary results on soft tissues are promising.

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